

Total Reducing Antioxidant Capacity of Thai Herbal Aromatic Powder (Ya-Hom) Measured by FRAP Assay

นิพนธ์ต้นฉบับ

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บทคัดย่อ

วัตถุประสงค์: เพื่อทดสอบความสามารถในการเป็นสารต้านออกซิเดชันของตำรับยาหอม 5 ตำรับ โดยวิธี FRAP **วิธีการศึกษา:** ทดสอบฤทธิ์ต้านออกซิเดชันของสารสกัดเหลวของผงยาหอม 5 ตำรับ โดยการวัด total phenolic compounds โดยใช้ Folin-Ciocalteu reagent วัดฤทธิ์ต้านออกซิเดชันโดยวิธี FRAP assay แล้วแสดงเป็นค่า micromolar ของ Fe(II) ต่อน้ำหนักผงแห้ง 1 กรัม โดยเทียบกับสารที่เป็น reducing agent มาตรฐานสองชนิด คือ ascorbic acid และ วิตามินอี ชนิดละลาย (Trolox®) **ผลการศึกษา:** พบปริมาณ total phenolic compounds ในแต่ละตำรับในระดับปานกลางถึงสูง ตามปริมาณ gallic acid equivalent (GAE) ที่พบค่าจาก 17.6 ± 4.3 ถึง 50.8 ± 10.9 มก. ต่อมผงแห้ง 1 กรัม พบว่าค่า FRAP มีค่าตั้งแต่ 2040 ± 140 ถึง 7110 ± 136 micromolar ต่อมผงแห้ง 1 กรัม ค่า ascorbic acid equivalent พบตั้งแต่ 123 ± 16 ถึง 401 ± 82 micromol ต่อมผงแห้ง 1 กรัม และค่า Trolox equivalent ตั้งแต่ 100 ± 5 ถึง 353 ± 69 micromol ต่อมผงแห้ง 1 กรัม **สรุป:** ตำรับยาหอม 5 ชนิดมีทั้ง total phenolic compounds และค่า FRAP ในระดับสูง ซึ่งค่า FRAP ที่สูงชี้แนะว่าน่าจะมีศักยภาพในการพัฒนาเป็นผลิตภัณฑ์ธรรมชาติเพื่อต้านออกซิเดชัน

คำสำคัญ: ยาหอม, ฤทธิ์ต้านออกซิเดชัน, FRAP, total phenolic compounds

Abstract

Objective: To investigate the total reducing antioxidant capacity of five brands of Ya-hom preparations using FRAP method. **Methods:** Aqueous extracts of five Thai herbal aromatic powder (Ya-hom) preparations were tested for the total phenolic compounds using Folin-Ciocalteu reagent. The antioxidant activity was examined using FRAP assay. FRAP values were expressed as micromolar of Fe(II)/g of dry powder. The total reducing capacity was compared to two standard reducing agents, ascorbic acid and a synthetic water soluble vitamin E (Trolox®). **Results:** The total phenolic compounds of each preparation resulted in moderate to high levels of gallic acid equivalent values varying from 17.6 ± 4.3 to 50.8 ± 10.9 mg/g dry powder. The FRAP values were found to range from 2040 ± 140 to 7110 ± 136 micromolar/g dry powder. The ascorbic acid equivalent ranged from 123 ± 16 to 401 ± 82 micromol/g dry powder and Trolox equivalent values varied from 100 ± 5 to 353 ± 69 micromol/g dry powder. **Conclusion:** This study suggests that the selected Ya-hom preparations contain high amounts of total phenolic compounds and high FRAP values. The high FRAP values may return a potential benefit as a natural antioxidant.

Keywords: Ya-hom, antioxidation, FRAP, total phenolic compounds

Introduction

Herbal aromatic powder or Ya-hom is a Thai traditional medicine which has long been used in Thailand. First two traditional formulas of Ya-hom (เทพจิตร์ and นาโกฐ) are listed as "National Herbal Medicine Products"¹. Commercial Ya-hom preparations in Thailand are available in different kinds and amount of medicinal plant compositions and exact components of most preparations are undisclosed. Although most Ya-hom preparations are different in recipes but the indication of all preparations are used for relief nausea, vomiting and as blood circulation stimulant. The cardiovascular effect of one preparation of Ya-hom was systematically studied to support the use for treatment of fainting²⁻⁴. The action for stomach discomfort treatment was studied and revealed that Ya-hom inhibited the secretion of acid, pepsin and soluble mucous induced by histamine and

carbachol in male Wistar rats⁵. Several studies in medicinal plants have been focused and revealed that they contain wide variety of natural antioxidants which are more potent than those from common dietary plants⁶⁻⁸. The phytochemicals that possess the antioxidant activity are phenolic compounds, flavonoids and tannins. Suweswaran et al.⁷ studied in 83 Ayurvedic medicinal plants and found that phenolic compounds in medicinal plants were the dominant antioxidant constituents. Other dietary foods from natural origins have been extensively investigated for the antioxidant activity to find out the potential benefits to counteract the free radicals which are well known as the causes of many degenerative diseases in human.⁹⁻¹¹ Since most Ya-hom preparations compose of large variety of medicinal plants therefore they should contain good proportion of botanical

ingredients which can provide antioxidant activity for the users. Yet, there is no data on antioxidant in Ya-hom preparations and it has become interesting to study.

Various assay methods have been developed to measure the antioxidant capacity. Ferric reducing antioxidant power (FRAP) assay is a simple and inexpensive measurement of the total reducing antioxidant capacity and has been used to analyze antioxidant capacity in plasma samples¹², marine organisms in ecotoxicological study¹³ and antioxidant activity in various medicinal plants¹⁴, food and beverages.¹⁵⁻¹⁷ FRAP assay is not a direct test for total antioxidant activity but it indirectly reflects the ability in reducing any reactive species by electron donation. Living organisms oxidize carbohydrate and fat to generate energy. Most free radicals in the body are produced by endogenous metabolism, actually oxidation. Other exogenous factors are ionizing radiation, pollution, injuries and diseases. Cells and tissues are naturally in reduced state relatively to the environment and maintain the normal reduced state by biological redox reactions.¹⁸ Vitamin C and glutathione are key reducing chemicals to counteract the major free radicals. When the available reducing agent power falls or oxidative stress rises, this condition damages the health¹⁹. In the present study, 5 brands of commercially accessible registered Ya-hom were tested for reducing capacity by ferric reducing antioxidant power assay to compare the additional benefit from the herbal blends.

Materials and Methods

Chemicals

Gallic acid, 2 M Folin-Ciocalteu reagent and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Fluka. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox[®]) was purchased from Aldrich. All other chemicals were of analytical grade. Five brands of Ya-hom (A, B, C, D, E) were purchased from various drugstores.

Ya-hom extraction

One gram of each brand of Ya-hom powder was macerated in a 100.0 ml of deionized water for 24 hours with occasional shaking and was filtered. The extracts were kept in 4°C until used. Two lots of each brand were extracted and tested in triplicate.

Determination of total phenolic compounds

An aliquot of 0.1 ml of the extract was mixed with 5.0 ml of 0.2 M Folin-Ciocalteu reagent for 5 min then 4.0 ml of 7.5% w/v sodium carbonate was added. The reaction tube was incubated in the dark for 30 mins at room temperature. The developed blue color was measured at 765 nm. Total phenolic compound of each sample was expressed as gallic acid equivalent (GAE) in mg/g dry powder based on the calibration curve conducted from the standard gallic acid solutions (10 - 60 mg%) and reacted in the same manner as the tested samples.

FRAP assay

FRAP reagent was freshly prepared by mixing the volume ratio of 10:1:1 of the following stock solutions respectively, 300 mM acetate buffer pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM FeCl₃.6H₂O solution. A 0.1 ml of the extract was mixed with 5.0 ml of FRAP reagent for 8 min in the dark. Absorbance of the ferrous-tripyridyltriazine (Fe(II)-TPTZ) complex blue color was read at 593 nm. The FRAP value was converted using standard curve of FeSO₄.7H₂O solution (500-2000 micromolar) and reacted with FRAP reagent in the same manner to build up a calibration curve of Fe(II)-TPTZ. Ascorbic acid (250-2500 micromolar) and Trolox[®] (100-1500 micromolar) were used to build up calibration curves of two standard antioxidants. All the solutions were reacted concomitantly using the same FRAP reagent.

Statistical analysis

Data were expressed as mean \pm SD. The correlation coefficient (R^2) between the parameter tested was established by regression analysis. Mean differences were analyzed by ANOVA. Post-hoc comparisons were made with Scheffe's test. Differences were considered significant at P -value < 0.05.

Results and Discussions

Total amount of phenolic compounds of all aqueous extracts were examined from a concomitantly constructed calibration curve of standard gallic acid solutions. The mean gallic acid equivalent values of all brands (A to D) were found to be fairly high varying from 17.6 ± 4.3 to 20.9 ± 1.0

mg/g dry powder, except for brand E that presented a significantly higher GAE values of 44.4 ± 5.1 and 50.8 ± 10.9 mg/g ($p < 0.05$) (as shown in Table 1). The GAE values among the two lots of all five brands were not significantly difference.

Table 1 The total phenolic compounds of Ya-hom powders (n = 3)

Brand	Gallic acid equivalent (mg/g dry powder) (mean \pm SD)	
	Lot 1	Lot 2
A	19.5 ± 4.1	19.2 ± 2.6
B	19.9 ± 0.8	20.9 ± 0.8
C	18.8 ± 1.8	20.9 ± 1.0
D	17.6 ± 4.3	18.6 ± 5.7
E	50.8 ± 10.9	44.4 ± 5.1

FRAP assay is an indirect method to investigate the reducing antioxidant power among the Ya-hom samples. In fact, the ferrous state of iron is harmful, known as a pro-oxidant that accelerates lipid peroxidation by breaking down hydrogen peroxide and lipid peroxide to reactive free radicals (ROS) via Fenton reaction ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH}^- + \cdot\text{OH}$)²⁰. At low pH, ferric-tripyridyltriazine (Fe(III)-TPTZ) complex (pale to colorless) was reduced in stoichiometric concern to ferrous form (Fe(II)-TPTZ) which has an intense blue color. FRAP assay is nonspecific that any half reaction having a lower redox potential than ferric-ferrous reaction, can change Fe(III) to Fe(II)¹². In the study, total phenolic compounds in the extracts act as reductants in a simple redox reaction. Thus, the higher in the absorbance, the higher in the total reducing power of the Ya-hom extract is reflected.

FRAP values were calculated using standard solutions of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ which reacted with the FRAP reagent to entail the resulting amount of Fe(II)-TPTZ reduced by the reductants in the samples. The ability of a compound to produce Fe(II) from Fe(III) defined as "antioxidant power"²¹. In fact, all reductants are not necessary antioxidants because a reductant may not reduce a pro-oxidant but the antioxidant reduces both the oxidant and pro-oxidant. Nevertheless, not all antioxidants can reduce ferric to ferrous. Thus, FRAP assay really reflects the antioxidant power in the tested samples. Ascorbic acid equivalent (AE) values and Trolox[®] equivalent (TE) values confirm the electron donating mechanism. Ascorbic acid is the most

powerful water soluble reducing agent in the body and Trolox[®], a synthetic vitamin E, of which structure is similar to alpha-tocopherol but lacks of hydrophobic tail, represents a lipid soluble antioxidant. Both were used as two standard antioxidants. Figure 1 shows a representative calibration curves for determining FRAP values, ascorbic acid and Trolox[®] equivalent values.

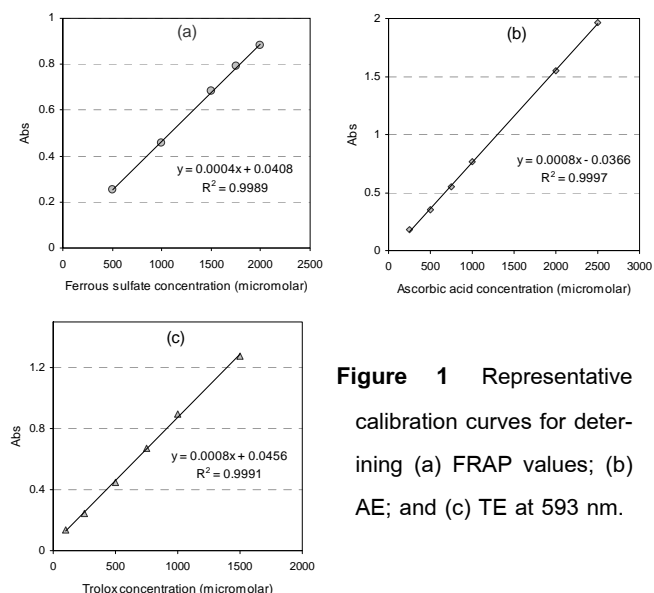


Figure 1 Representative calibration curves for determining (a) FRAP values; (b) AE; and (c) TE at 593 nm.

Table 2 Reducing antioxidant capacity of Ya-hom powders

Brand	FRAP value (micromolar/g)		AE (micromol/g)		TE (micromol/g)	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
A	2610 ± 130	2410 ± 130	152 ± 14	142 ± 17	129 ± 5	119 ± 5
B	2680 ± 470	2870 ± 530	158 ± 33	168 ± 38	132 ± 25	142 ± 28
C	2040 ± 140	2240 ± 120	123 ± 16	133 ± 24	100 ± 5	110 ± 6
D	2200 ± 550	2170 ± 540	131 ± 42	129 ± 43	108 ± 27	106 ± 26
E	7110 ± 1360	6750 ± 820	401 ± 82	383 ± 67	353 ± 69	335 ± 42

Table 2 shows the results of FRAP values of Fe(II)-TPTZ complex generated by the antioxidant in the samples. Ya-hom A to D resulted in comparable FRAP values from 2040 ± 140 to 2870 ± 530 micromolar/g dry powder, while brand E exhibited significantly higher FRAP values, 7110 ± 1360 and 6750 ± 820 micromolar/g dry powder for lot 1 and lot 2, respectively ($p < 0.05$).

Ya-hom extracts resulted moderate to high values of AE varied from 123 ± 16 to 401 ± 82 micromol/g and those of the TE varied from 100 ± 5 to 353 ± 69 micromol/g. Both AE and TE values are almost identical, according to the same antioxidant mechanism in reducing Fe(III)-TPTZ to Fe(II)-TPTZ by electron donation. Thus, it is negligible to use either ascorbic or Trolox[®] as a positive control in FRAP assay to compare the reducing power of the samples in molar unit.

Conclusion

The study has demonstrated that all selected Ya-hom preparations show noteworthy reducing power that implies a promising antioxidant capacity when taken in powder form. The FRAP values, AE and TE are supposed to rely on the amount of total phenolic compounds in the commercial recipes but the GAE values of the first four brands are almost equal, accordingly it can not achieve the correlation between the FRAP values and the GAE values. Nonetheless, this study is not entail any difference of the registered therapeutic efficacy of each preparation but just suggests that Ya-hom, the Thai traditional medicine, could provide moderate to high potential benefit as ancillary natural antioxidant to the users.

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Editorial note

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